TITLE: Role of cyclooxygenase in the vascular responses to extremity cooling in Caucasian and African males

AUTHORS: Matthew J Maley[1,2], James R House[1], Michael J Tipton[1] and Clare M Eglin[1]

RUNNING TITLE: Extremity cooling and COX inhibition in Caucasians and Africans

AFFILIATION: [1]Extreme Environments Laboratory, Department of Sport and Exercise Science, University of Portsmouth, PO1 2ER, UK. [2]Institute of Health and Biomedical Innovation, School of Exercise and Nutrition Sciences, Queensland University of Technology, 4059, Australia.

CORRESPONDING AUTHOR: M J Maley, Institute of Health and Biomedical Innovation, School of Exercise and Nutrition Sciences, Queensland University of Technology, 4059, Australia. Email: matthew.maley@qut.edu.au

KEYWORDS: Non-freezing cold injury, ethnicity, extremity cooling, cyclooxygenase

WORD COUNT: 3820

NUMBER OF REFERENCES: 55

SUBJECT AREA: Human / environmental and exercise physiology

NEW FINDINGS

WHAT IS THE CENTRAL QUESTION OF THIS STUDY?

Compared with Caucasians, African individuals are more susceptible to non-freezing cold injury and experience greater cutaneous vasoconstriction and cooler finger skin temperatures upon hand cooling. We investigated whether the enzyme cyclooxygenase is, in part, responsible for the exaggerated response to local cooling.

WHAT IS THE MAIN FINDING AND ITS IMPORTANCE?

During local hand cooling, African descent individuals experienced significantly lower finger skin blood flow and skin temperature compared with Caucasians irrespective of cyclooxygenase inhibition. These data suggest that in young African males the cyclooxygenase pathway appears not to be the primary reason for the increased susceptibility to non-freezing cold injury.

ABSTRACT

African descent (AFD) individuals are more susceptible to non-freezing cold injury (NFCI) and experience an exaggerated cutaneous vasoconstrictor response to hand cooling compared with Caucasians (CAU). Using a placebo-controlled, crossover design, this study tested the hypothesis that cyclooxygenase (COX) may, in part, be responsible for the exaggerated vasoconstrictor response to local cooling in AFD. Twelve AFD and twelve CAU young healthy males completed foot cooling and hand cooling (separately, in 8 °C water for 30 minutes) with spontaneous rewarming in 30 °C air following placebo or aspirin (COX inhibition) treatment. Skin blood flow, expressed as cutaneous vascular conductance (CVC, flux•mmHg^{-1}), and skin temperature (T_{sk}) were measured throughout. Irrespective of COX inhibition, the responses to foot cooling, but not hand cooling, were similar between ethnicities. Specifically, during hand cooling following placebo, AFD experienced a lower minimum skin blood flow (0.5 [0.1] *vs.* 0.8 [0.2] flux•mmHg^{-1}, $P < 0.001$) and a lower minimum finger T_{sk} (9.5 [1.4] °C *vs.* 10.7 [1.3] °C, $P = 0.039$) compared with CAU. During spontaneous rewarming average skin blood flow was also lower in AFD than CAU (2.8 [1.6] *vs.* 4.3 [1.0] flux•mmHg^{-1}, $P < 0.001$). These data provide further support that AFD experience an exaggerated response to hand cooling and thus NFCI; however, the results demonstrate the COX pathway is not the primary reason for the exaggerated responses in AFD and increased susceptibility to NFCI.

LIST OF ABBREVIATIONS

AFD African descent

AUC Area under curve

CAU Caucasian

COX Cyclooxygenase

1	CVC	Cutaneous vascular conductance
2	IQR	Interquartile range
3	LDU	Laser Doppler units
4	MAP	Mean arterial pressure
5	NFCI	Non-freezing cold injury
6	ROS	Reactive oxygen species
7	TXA_2	Thromboxane A_2

INTRODUCTION

On initial whole-body cold exposure there is an immediate decrease in skin temperature (T_{sk}) which elicits local and centrally-induced constriction of the cutaneous circulation which serves as a defence mechanism, preventing heat loss from the body in an attempt to delay the onset of hypothermia (Spealman, 1945; Granberg, 1991; Kenney & Armstrong, 1996). In exchange for the protection of deep body temperature an individual is at risk of other cold injuries such as non-freezing cold injury (NFCI), with the feet more susceptible than the hands (Ungley & Blackwood, 1942). Symptoms of this injury often include pain, whilst mild cooling of the affected extremity is associated with an exaggerated vasoconstrictor response and slow rewarming which may leave an individual prone to further cold injuries. These symptoms may last for the remaining lifetime of an individual (Ungley et al., 1945).

In the military population, particularly during conflict, cold injuries have resulted in hundreds of thousands of casualties (Paton, 2001; Golden et al., 2013). In the UK Armed Forces, between 2006 and 2016, there were 1,235 successful personnel compensation claims for NFCI, with the most recent year (March 2015–2016) costing the Ministry of Defence £1,486,200. However, NFCI is not solely a military problem as cases have been reported in civilians (Ramstead et al., 1980; Wrenn, 1991) and those who expose themselves to cold during leisure activities, such as mountaineering or cold expeditions (O'Brien & Frykman, 2003). Recently 'Sport England' (2016) reported nearly 1.5 million individuals participate each month in sports which may involve cold exposure such as angling, water sports, mountaineering and snow sports, therefore the risk of NFCI is ever-present.

A major predisposing risk factor on the incidence of NFCI is ethnicity. Individuals of African descent (AFD) are more susceptible to cold injuries compared with Caucasian individuals (CAU) under the same environmental conditions (DeGroot et al., 2003; Burgess & Macfarlane, 2009). Within the United States Armed Forces between 2009 and 2014 there were 3,008 individuals who suffered a cold injury, with AFD injured at twice the rate of CAU (Connor, 2014). Despite increased awareness and education, NFCI and other cold injuries remain a current issue.

The mechanism(s) for the increased incidence of NFCI in AFD is not clear at present. During local hand cooling (8 °C for 30 minutes) and spontaneous rewarming we have previously observed that AFD experience greater vasoconstriction and also rewarm later and more slowly than CAU (Maley et al., 2014). The comparatively lower skin blood flow and lower T_{sk} in AFD may explain the increased susceptibility to NFCI. In addition we have shown that AFD appear to have a reduced endothelial function, showing a smaller vasodilatory response to ACh compared with CAU (Maley et al., 2015, 2017); however, the reason for the differences in response to local cooling remains unclear.

During local cooling the decrease in skin blood flow is a result of sympathetic adrenergic activation releasing noradrenaline acting on post-synaptic adrenergic receptors (Pergola et al., 1993; Johnson et al., 2005), as well as an inhibition of nitric oxide production and contributions from non-adrenergic pathways (Hodges et al., 2006; Thompson-Torgerson et al., 2007; Johnson et al., 2014). Furthermore, cooling increases reactive oxygen species (ROS) (Bailey et al., 2005) which, along with reducing the bioavailability of nitric oxide, leads to translocation of α_{2C} receptors to the cell surface, capable of exaggerating the response to local cooling (Chotani et al., 2000, 2004). Compared with CAU, *in vivo* and *in vitro* data demonstrates AFD experience heightened oxidative stress and reduced bioavailability of nitric oxide (Kalinowski et al., 2004; Feairheller et al., 2011). This oxidative stress may be produced from various sources such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and cyclooxygenase (COX) (Kukreja et al., 1986; Griendling & FitzGerald, 2003; Vanhoutte, 2011). Along with other prostanoids, COX produces TXA_2, a vasoconstrictor which plays a role in the cold-induced vasoconstriction in response to hand cooling in those with Raynaud's phenomenon (Tindall et al., 1985), demonstrating TXA_2 may play a role in those with altered vascular function. Coupled with the information that AFD experience greater levels of oxidative stress, perhaps produced through COX, and that COX increases ROS and produces TXA_2, it is possible that this enzyme may contribute to the accentuated vasoconstrictor response in AFD during local cooling.

Therefore, the aim of the present study was to determine whether the accentuated vasoconstrictor response during local cooling and the delayed release during rewarming in AFD involves the COX pathway. It was hypothesised that COX inhibition would

attenuate the local cooling induced vasoconstriction and result in AFD experiencing local vascular responses similar to CAU.

METHODS

This study was given a favourable ethical opinion from the University of Portsmouth Science Faculty Ethics Committee and complied with standards set in 'The Declaration of Helsinki' (October 2013). The participants were made aware of the purpose, procedures and risks of the study prior to giving their informed written consent. Twelve CAU and 12 AFD male volunteers participated in the study; their physical characteristics are presented in Table 1.

All CAU were born in the UK. Eight AFD were born in the UK whilst four were born in Africa (Zimbabwe, Ghana, Kenya and Uganda) and had resided in the UK for an average of 11 years, with a minimum of seven years. All participants were non-smokers, were free from asthma and any vascular or blood disorders including hypertension, diabetes and Raynaud's phenomenon, with no history of either freezing or non-freezing cold injuries. Participants' history of cold exposure was ascertained by questionnaire with each ethnic group reporting similar exposure to cold. Ethnicity was determined by self-classification. To minimise heterogeneity, participants were only recruited if both parents and all four grandparents had known to originate from the same ethnic group. Prior to testing, participants were asked to refrain from consuming alcohol for 24 hours and participating in exercise or consuming caffeine for 12 hours.

EXPERIMENTAL PROCEDURES AND MEASUREMENTS

In a balanced, cross-over, placebo controlled design, participants were required to attend the laboratory on two separate occasions at the same time of day. Both visits included immersion of the foot and hand, separately, into cold water. As we previously observed no ethnic differences in T_{sk} and skin blood flow between ethnic groups in response to foot immersion in 15 °C water for two minutes (Maley et al., 2014), we decided to employ a cold-induced vasodilatation (CIVD) protocol, previously utilised (Maley et al., 2014), consisting of immersion in water at 8 °C for 30 minutes for both the foot and hand cooling. Previous research has demonstrated greater reactivity and comfort to 8 °C water compared with colder water (Mekjavic et al., 2013; Tyler et al., 2015). For every participant, foot cooling was performed first followed by hand cooling.

Immediately prior to entering the climate controlled chamber participants were asked to consume 150 mL of diluted orange squash with or without soluble aspirin (acetylsalicylic acid; 600 mg, Boots Inc., UK), blinded to the participant. Aspirin irreversibly inhibits COX by acetylation of the active site of COX (Vane, 1971) with this dose of aspirin shown to inhibit 86 % of bradykinin-induced production of prostacyclin and 99 % inhibition of TXA_2 production by platelets at 30 minutes (Heavey et al., 1985).

Participants entered the climate controlled chamber (mean [SD] T_{db}: 30.3 [0.9] °C, T_{wb}: 22.9 [0.9] °C, WBGT: 25.1 [0.9] °C) removed their socks and shoes and rested in a semi-recumbent position for 25 minutes whilst being instrumented. A laser Doppler probe (VP1T / 7, Moor Instruments, UK) was applied to the Great toe of the right foot using double sided adhesive rings to measure skin blood flow using a laser Doppler flowmetry monitor (moorVMS-LDF, Moor Instruments, UK). Laser Doppler data were recorded using a data acquisition system and software (Powerlab and LabChart 7, AD Instruments, New Zealand). A thermistor (Type EUS-U, Grant Instruments, UK) was applied to the medial aspect of the Great toe pad of the right foot using a single piece of adhesive tape. T_{sk} was measured using a squirrel 1200 series data logger (Grant Instruments, UK) and recorded every five seconds.

Following the 25-minute rest period participants placed their right foot into a plastic bag and immersed it up to the point of the mid-malleoli into a stirred water bath (Grant Instruments, UK) maintained at 35.0 (0.3) °C for five minutes. Following this, participants removed their foot, still within the plastic bag, and immediately placed it in a stirred water bath maintained at 8.2 (0.1) °C for a further 30 minutes. After foot cooling, participants removed their foot from the water bath and plastic bag to allow spontaneous rewarming of the foot for 15 minutes. Following this, participants were given 150 mL of diluted orange squash with or without soluble aspirin (300 mg). The reason for adding an extra dose of aspirin is due to the return of endothelial COX function within 90 minutes and to ensure that COX function was inhibited as much as possible (Heavey et al., 1985). If a participant received aspirin at the beginning of the protocol they also received aspirin in their second drink; if the participant was not given aspirin in their first drink, then they were not given aspirin in their second drink (Fig. 1).

Within the next 25 minutes participants were instrumented for their hand immersion protocol. A laser Doppler probe and skin thermistor were placed onto participant's middle and second finger, respectively, of the right hand. Participants then placed their hand into a plastic bag and immersed it to the styloid process in a water bath maintained at 35.0 (0.2) °C for five minutes. The protocol for the hand was the same as for the foot in that cooling (8.1 [0.1] °C) and spontaneous rewarming followed. Blood pressure was measured from the left arm, using an automated monitor (Minimon 7137 Plus, Kontron Instruments, UK), for both the foot and hand cooling protocols and recorded two minutes prior to cooling, 30 seconds, five and 15 minutes into cooling, followed by 30 seconds and five minutes into rewarming.

Participants' height and mass was measured using a stadiometer (Leicester, Bodycare, UK) and digital weighing scales (Ohaus I-10, Ohaus Corporation, USA), respectively. Length of participants' hand and foot was measured using a segmometer (Segmometer 4, Rosscraft, Canada). Hand and foot volume was calculated using a water displacement method.

DATA ANALYSES

As mean arterial pressure (MAP = ((2*diastolic)+systolic) / 3) differed over time (see results), skin blood flow was calculated as cutaneous vascular conductance (CVC) (laser Doppler units / MAP = CVC (flux•mmHg^{-1})). T_{sk} was plotted every five minutes and, from the sampled data which was recorded every five seconds, the CIVD variables were determined which included: average T_{sk}, minimum T_{sk}, number of CIVD waves, onset time of first CIVD wave, T_{sk} prior to onset of first CIVD wave and maximum T_{sk} during the first CIVD wave and amplitude of CIVD wave (Fig. 2). For the skin blood flow assessment during local cooling and rewarming, average, minimum and area under the curve (AUC, calculated using GraphPad, Version 5, USA) were calculated.

Statistical analyses were conducted using IBM SPSS for Windows version 20 (IBM SPSS Statistics, USA). Data was assessed for normality with a Shapiro-Wilk test. An α of 0.05 was used to determine statistical significance. Participant characteristics were analysed using an independent samples *t*-test. MAP throughout immersion was analysed between- and within-groups with a repeated measures ANOVA to identify any differences in blood pressure between ethnicities and between COX inhibition and

placebo. Where a significant main effect was observed (see results), pairwise comparisons were carried out and adjusted for multiple comparisons using the Holm-Bonferroni procedure.

Baseline CVC (i.e. final minute of warm water immersion) between- and within-groups was analysed using an independent and paired samples *t*-test, respectively. Baseline T_{sk} (i.e. final five seconds of warm water immersion) between- and within-groups was analysed using a Mann-Whitney U test and a Wilcoxon Signed Rank test, respectively. CIVD variables, CVC average, minimum and AUC during cooling and rewarming between-groups were analysed using either an independent samples *t*-test or a Mann-Whitney U test. The same variables within-groups were analysed using a paired samples *t*-test or a Wilcoxon Signed Rank test. Between- and within-groups CVC and T_{sk} throughout immersion was analysed using a Mann-Whitney U and Wilcoxon Signed Rank Test, respectively, and adjusted for multiple comparisons using the Holm-Bonferroni procedure.

Parametric data in text is presented as mean (SD) with non-parametric data presented as median and interquartile range (IQR). Figures shown are presented as mean (SD). Effect sizes, where appropriate, were calculated and are denoted by *d* for parametric data and *r* for non-parametric data.

RESULTS

PARTICIPANTS

All participant characteristics were similar ($P > 0.05$), except AFD had a longer hand length ($P = 0.018$, $d = 1.04$) and a greater hand volume ($P = 0.043$, $d = 0.87$) than CAU.

MEAN ARTERIAL PRESSURE

There was no main effect for ethnicity or condition (i.e. placebo *vs.* COX inhibition) for both foot or hand immersion protocols (mean [SD], baseline placebo, foot, CAU: 83 [5] mmHg, AFD: 83 [7] mmHg; hand, CAU: 82 [7] mmHg, AFD: 81 [8] mmHg, $P > 0.05$). However, there was a main effect for time for both the foot ($P < 0.001$) and hand ($P = 0.022$) protocol with pairwise comparisons showing MAP being elevated for the first five minutes of immersion in the foot protocol only (Table 2). There was no statistical

interaction between condition, time and ethnicity for both immersion protocols (P > 0.05).

BASELINE SKIN BLOOD FLOW AND SKIN TEMPERATURE

Following COX inhibition, CAU had lower baseline toe CVC (Table 3, P = 0.019, d = 1.03) and T_{sk} (Table 3, P = 0.045, r = 0.41) than AFD. Otherwise, baseline values were similar (Table 3).

FOOT RESPONSES TO COOLING AND SUBSEQUENT REWARMING

SKIN BLOOD FLOW

Toe skin blood flow during foot cooling and rewarming was similar between- and within-groups following placebo and COX inhibition (Table 4, Fig. *3A*, P > 0.05).

SKIN TEMPERATURE

There was a tendency for CAU to have a lower average T_{sk} compared with AFD following COX inhibition (Table 5, P = 0.052, d = 0.84). Following placebo, only two CAU and four AFD experienced CIVD. Following COX inhibition, only two CAU and seven AFD experienced CIVD. Therefore, analyses on these variables could not be conducted.

Toe T_{sk} during foot cooling and rewarming was similar between- and within-groups following placebo and COX inhibition (Fig. 3*B*, P > 0.05).

FINGER RESPONSES TO COOLING AND SUBSEQUENT REWARMING

SKIN BLOOD FLOW

During cooling, COX inhibition resulted in a higher minimum CVC in AFD compared with placebo (Table 6, P = 0.039, d = 0.78). During cooling, following placebo, minimum CVC was lower in AFD compared with CAU (P < 0.001, d = 2.03) whilst there was a tendency for average CVC (P = 0.050, d = 0.84) and AUC (P = 0.053, d = 0.83) to be lower in AFD compared with CAU. During rewarming AFD had lower average CVC (P = 0.009, d = 1.17) and AUC (P = 0.009, d = 1.18) compared with CAU.

During cooling, following COX inhibition, there was a tendency for average CVC (P = 0.052, d = 0.85) and AUC (P = 0.055, d = 0.84) to be lower in AFD compared with CAU.

During rewarming AFD experienced a lower average CVC ($P < 0.001$, $d = 1.69$), minimum CVC ($P = 0.017$, $d = 1.05$) and AUC ($P = 0.001$, $d = 1.63$) compared with CAU.

During cooling and subsequent rewarming finger skin blood flow was lower in AFD than CAU following both placebo and COX inhibition (Fig. 4*A*).

SKIN TEMPERATURE

For CAU, COX inhibition resulted in a warmer average finger T_{sk} (Table 7, $P = 0.049$, $r = 0.28$) and a faster onset time of CIVD ($P = 0.035$, $r = 0.13$) compared with placebo. Minimum finger T_{sk} was lower in AFD compared with CAU following both placebo ($P = 0.004$, $d = 1.30$) and COX inhibition ($P = 0.034$, $d = 0.92$).

Finger T_{sk} during cooling and rewarming was lower in AFD compared with CAU following both placebo and COX inhibition (Fig. 4*B*).

DISCUSSION

The primary findings of this study were *1)* during foot cooling, toe skin blood flow (Fig. 3*A*) and T_{sk} (Fig. 3*B*) did not differ between ethnic groups irrespective of COX inhibition, and *2)* during local hand cooling AFD experienced significantly lower finger skin blood flow (Fig. 4*A*) and T_{sk} (Fig. 4*B*) compared with CAU irrespective of COX inhibition. Following placebo, the present study confirms previous findings between CAU and AFD in response to hand cooling (Iampietro et al., 1959; Newman, 1969; Jackson et al., 1989; Maley et al., 2014), whilst the responses to foot cooling (8 °C for 30 minutes) are the first to be reported here. Contrary to our hypothesis these data suggest that in young AFD healthy males the COX pathway appears not to be the primary reason for the increased susceptibility to NFCI.

During foot cooling AFD responded in a comparable manner to CAU. Given that NFCI is more prominent in the feet and AFD are more susceptible to NFCI, it was expected that CAU would respond to foot cooling with warmer T_{sk} and greater skin blood flow responses compared with AFD. The authors are not sure why no ethnic differences were observed in the direction expected. Thickness in skin may be a contributing factor as the authors noted that the skin on the sole of the foot in African descent participants

felt ‘thicker’. Whilst the stratum corneum thickness is similar between African and European individuals on skin sites such as the hip, arm and face (Thomson, 1955; Freeman et al., 1962; Richards et al., 2003), the deeper dermis layer is thicker in AFD (Richards et al., 2003). If the skin (epidermis or dermis) is thicker on the sole of the foot in African individuals then this may have provided some protection (insulation) from the cold stimulus and hidden a dysfunctional microvascular response. Stratum corneum thickness has been discussed previously as a factor that may influence finger cooling rates (Jay & Havenith, 2004). However, as previously observed (Maley et al., 2014), it is possible that AFD do not experience a dysfunctional response to local foot cooling without centrally-driven vasoconstriction.

Interestingly, during foot cooling some participants experienced a very strong local vasoconstrictor response, whilst other exhibited only slight changes. The reason for the variability is not known. Individuals born in a tropical climate may experience exaggerated responses to local finger cooling (Lee et al., 2013), however eight out of the 12 participants in this study were born in the UK, with the four born outside the UK not primarily responsible for the variability. While participants reported similar exposure to cold, other factors, such as differences in deep body temperature (Daanen et al., 1997) and physical fitness (Keramidas et al., 2010), may have contributed to the variability in responses.

The present study does not lend support to a previous pilot study (Belvins et al., 2014) where it was suggested that COX inhibition in CAU may facilitate local skin blood flow during foot cooling and spontaneous rewarming. The present study employed a colder water temperature (8 °C *vs.* 15 °C) and one immersion of 30 minutes in comparison to three repeated immersions of ten minutes in the pilot study. Different cooling rates and duration between the two studies may have contributed to confliction in results, however further elaboration of differences between studies is not possible at present given the little information available.

Using forearm and finger venous occlusion plethysmography, previous research has demonstrated the reflex vascular responses to arm or foot cooling are not altered by COX inhibition (Cowley et al., 1983, 1985; Gresele et al., 1985; Hechtman & Jageneau, 1985), with one study demonstrating an increase in vascular resistance during foot

cooling (Serneri et al., 1981). As the present study observed the local vascular response (using laser Doppler flowmetry) to local cooling, the results are difficult to compare to previous studies as reflex vasoconstriction is controlled differently to local vasoconstriction (Johnson et al., 2014).

As hypothesised, during hand immersion, the fingers of AFD reached a lower temperature and rewarmed more slowly than CAU, confirming our previous findings (Maley et al., 2014). We also hypothesised that COX inhibition in AFD would result in warmer finger T_{sk} during hand cooling and spontaneous rewarming making their response similar to CAU, although this did not occur. As alluded to within the introduction, we hoped that COX inhibition would attenuate the production of ROS and in turn reduce the vasoconstriction experienced by AFD during local hand cooling. However, the present study appears to provide evidence that COX is not the major cause for the greater vasoconstrictor response and cooler T_{sk} to local hand cooling in AFD. The authors can only speculate that other sources of oxidative stress, such as NADPH oxidase (Griendling & FitzGerald, 2003), are partly responsible for the exaggerated responses experienced by AFD. Anti-oxidants capable of scavenging oxygen-derived free radicals prior to cooling should be investigated in future studies.

Despite COX inhibition not providing any advantage to AFD during local hand cooling, it was noted that CAU experienced a warmer average finger T_{sk} and earlier CIVD onset time (Table 7) following COX inhibition *versus* placebo. The difference in average finger T_{sk} was 0.6 °C and only four participants were included in the analyses for the CIVD onset time, therefore caution should be exercised when interpreting the present results. Additionally, it should be noted that COX inhibition use (e.g. acetaminophen) prior to whole-body cooling may lead to a faster decline in deep body temperature (Foster et al., 2016). While COX inhibition may produce warmer finger T_{sk} during local cooling, the authors do not recommend administering aspirin, or any other COX inhibitor, to an individual susceptible to NFCI.

Factors other than local cutaneous vasculature control may play a role in the increased susceptibility to NFCI in AFD. Previous research has shown AFD compared with CAU experience a lower deep body temperature, finger T_{sk} and oxygen uptake during whole-body cooling (Rennie & Adams, 1957; Adams & Covino, 1958; Farnell et al., 2008).

Given that the CIVD responses are blunted with colder environments / lower deep body temperatures (Keatinge, 1957; Sawada et al., 2000; Dobnikar et al., 2009) it is possible that factors such as thermogenesis and / or centrally-driven vasoconstriction, in combination with a depressed CIVD reaction (at least in the hands) during whole-body cooling may play a role in the increased susceptibility to NFCI in AFD. Further studies are being conducted to elucidate the extremity vascular responses to whole-body cooling between CAU and AFD.

It is concluded that young, healthy, male AFD and CAU show similar physiological responses to foot cooling. In contrast, compared with CAU, AFD experience lower finger skin blood flow and cooler finger T_{sk} in response to hand cooling irrespective of COX inhibition. Therefore, it is unlikely that the COX pathway is responsible for the increased susceptibility to NFCI in AFD.

REFERENCES

Adams T & Covino BG (1958). Racial variations to a standardized cold stress. *J Appl Physiol* 12**,** 2–5.

Anonymous (2016). Who plays sport? *Sport Engl*. Available at: http://www.sportengland.org/research/who-plays-sport/by-sport/ [Accessed June 20, 2016].

Bailey SR, Mitra S, Flavahan S & Flavahan NA (2005). Reactive oxygen species from smooth muscle mitochondria initiate cold-induced constriction of cutaneous arteries. *Am J Physiol Heart Circ Physiol* 289**,** 243–250.

Belvins W, Tipton M & Marshall JM (2014). Experimental investigation of non-freezing cold-induced injury (NFCI): are young Asian males more susceptible than young white Caucasians and are cyclooxygenase (COX) products involved? *FASEB J* 28**,** 1106.

Burgess JE & Macfarlane F (2009). Retrospective analysis of the ethnic origins of male British Army soldiers with peripheral cold weather injury. *J R Army Med Corps* 155**,** 11–15.

Chotani MA, Flavahan S, Mitra S, Daunt D & Flavahan NA (2000). Silent alpha(2C)-adrenergic receptors enable cold-induced vasoconstriction in cutaneous arteries. *Am J Physiol Heart Circ Physiol* 278**,** H1075–H1083.

Chotani MA, Mitra S, Su BY, Flavahan S, Eid AH, Clark KR, Montague CR, Paris H, Handy DE & Flavahan NA (2004). Regulation of alpha(2)-adrenoceptors in human vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 286**,** H59–H67.

Connor R (2014). Update: cold weather injuries, active and reserve components, U.S. Armed Forces, July 2009-June 2014. *Med Surveill Mon Rep* 21**,** 14–19.

Cowley AJ, Jones EW, Carter AJ, Hanley SP & Heptinstall S (1985). Effects of dazoxiben, an inhibitor of thromboxane synthetase, on cold-induced forearm vasoconstriction and platelet behaviour in different individuals. *Br J Clin Pharmacol* 19**,** 1–8.

Cowley AJ, Jones EW & Hanley SP (1983). Effects of dazoxiben, an inhibitor of thromboxane synthetase, on forearm vasoconstriction in response to cold stimulation, and on human blood vessel prostacyclin production. *Br J Clin Pharmacol* 15**,** 107S–112S.

Daanen HA, Van De Linde FJG, Romet TT & Ducharme MB (1997). The effect of body temperature on the hunting response of the middle finger skin temperature. *Eur J*

Appl Physiol Occup Physiol 76**,** 538–543.

DeGroot DW, Castellani JW, Williams JO & Amoroso PJ (2003). Epidemiology of U.S. Army cold weather injuries, 1980-1999. *Aviat Space Environ Med* 74**,** 564–570.

Dobnikar U, Kounalakis SN & Mekjavic IB (2009). The effect of exercise-induced elevation in core temperature on cold-induced vasodilatation response in toes. *Eur J Appl Physiol* 106**,** 457–464.

Farnell GS, Pierce KE, Collinsworth TA, Murray LK, Demes RN, Juvancic-Heltzel JA & Glickman EL (2008). The influence of ethnicity on thermoregulation after acute cold exposure. *Wilderness Environ Med* 19**,** 238–244.

Feairheller DL, Park J-Y, Sturgeon KM, Williamson ST, Diaz KM, Veerabhadrappa P & Brown MD (2011). Racial differences in oxidative stress and inflammation: in vitro and in vivo. *Clin Transl Sci* 4**,** 32–37.

Foster J, Mauger A, Thomasson K, White S & Taylor L (2016). Effect of acetaminophen ingestion on thermoregulation of normothermic, non-febrile humans. *Front Pharmacol* 7**,** 1–8.

Freeman RG, Cockerell EG, Armstrong J & Knox JM (1962). Sunlight as a factor influencing the thickness of epidermis. *J Investiative Dermatology* 39**,** 295–298.

Golden FSC, Francis TJR, Gallimore D & Pethybridge R (2013). Lessons from history: morbidity of cold injury in the Royal Marines during the Falklands Conflict of 1982. *Extrem Physiol Med* 2**,** 1–12.

Granberg P (1991). Human physiology under cold exposure. *Artic Med Res* 50**,** 23–27.

Gresele P, Bounameaux H, Arnout J, Perez-Requejo J, Deckmyn H & Vermylen J (1985). Thromboxane A2 and prostacyclin do not modulate the systemic hemodynamic response to cold in humans. *J Lab Clin Med* 106**,** 534–541.

Griendling KK & FitzGerald GA (2003). Oxidative stress and cardiovascular injury part I: Basic mechanisms and in vivo monitoring of ROS. *Circulation* 108**,** 1912–1916.

Heavey DJ, Barrow SE, Hickling NE & Ritter JM (1985). Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature* 318**,** 186–188.

Hechtman D & Jageneau A (1985). Inhibition of cold-induced vasoconstriction with ketanserin. *Microvasc Res* 30**,** 56–62.

Hodges GJ, Zhao K, Kosiba WA & Johnson JM (2006). The involvement of nitric oxide in the cutaneous vasoconstrictor response to local cooling in humans. *J Physiol* 574**,** 849–857.

Iampietro PF, Goldman ER, Buskirk ER & Bass DE (1959). Response of negro and white males to cold. *J Appl Physiol* 14**,** 798–800.

Jackson RL, Roberts DE, Cote RA, McNeal P, Faye JT, Sharp MW, Kraus E, Rahman SA & Hamlet MP (1989). *Psychological and physiological responses of blacks and caucasians to hand cooling*. Natick, Massachusetts. Available at: http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA215646.

Jay O & Havenith G (2004). Finger skin cooling on contact with cold materials: an investigation of male and female responses during short-term exposures with a view on hand and finger size. *Eur J Appl Physiol* 93**,** 1–8.

Johnson JM, Minson CT & Kellogg DL (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Compr Physiol* 4**,** 33–89.

Johnson JM, Yen TC, Zhao K & Kosiba WA (2005). Sympathetic, sensory, and nonneuronal contributions to the cutaneous vasoconstrictor response to local cooling. *Am J Physiol Heart Circ Physiol* 288**,** 1573–1579.

Kalinowski L, Dobrucki IT & Malinski T (2004). Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. *Circulation* 109**,** 2511–2517.

Keatinge WR (1957). The effect of general chilling on the vasodilator response to cold. *J Physiol* 139**,** 497–507.

Kenney WL & Armstrong CG (1996). Reflex peripheral vasoconstriction is diminished in older men. *J Appl Physiol* 80**,** 512–515.

Keramidas ME, Musizza B, Kounalakis SN & Mekjavic IB (2010). Enhancement of the finger cold-induced vasodilation response with exercise training. *Eur J Appl Physiol* 109**,** 133–140.

Kukreja RC, Kontos HA, Hess ML & Ellis EF (1986). PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. *Circ Res* 59**,** 612–619.

Lee J-Y, Bakri I, Matsuo A & Tochihara Y (2013). Cold-induced vasodilation and vasoconstriction in the finger of tropical and temperate indigenes. *J Therm Biol* 38**,** 70–78.

Maley MJ, Eglin CM, House JR & Tipton MJ (2014). The effect of ethnicity on the vascular responses to cold exposure of the extremities. *Eur J Appl Physiol* 114**,** 2369–2379.

Maley MJ, House JR, Tipton MJ & Eglin CM (2015). Vascular responses of the extremities to transdermal application of vasoactive agents in Caucasian and

African descent individuals. *Eur J Appl Physiol* 115**,** 1801–1811.

Maley MJ, House JR, Tipton MJ & Eglin CM (2017). Role of cyclooxygenase in the vascular response to locally delivered acetylcholine in Caucasian and African descent individuals. *Microvasc Res* 111**,** 80–87.

Mekjavic IB, Dobnikar U & Kounalakis SN (2013). Cold-induced vasodilatation response in the fingers at 4 different water temperatures. *Appl Physiol Nutr Metab* 38**,** 14–20.

Newman RW (1969). Cold acclimation in negro Americans. *J Appl Physiol* 27**,** 316–319.

O'Brien C & Frykman PN (2003). Peripheral responses to cold: case studies from an Arctic expedition. *Wilderness Environ Med* 14**,** 112–119.

Paton BC (2001). Cold, casualties, and conquests: the effects of cold on warfare. In *Medical Aspects of Harsh Environments. Volume 1*, ed. Pandolf KE & Burr RE, pp. 313–349. TMM Publications, Washington, D.C.

Pergola PE, Kellogg DL, Johnson JM, Kosiba WA & Solomon DE (1993). Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am J Physiol* 265**,** H785–H792.

Ramstead KD, Hughes RG & Webb AJ (1980). Recent cases of trench foot. *Postgrad Med J* 56**,** 879–883.

Rennie D & Adams T (1957). Comparative thermoregulatory responses of negroes and white persons to acute cold stress. *J Appl Physiol* 11**,** 201–204.

Richards GM, Oresajo CO & Halder RM (2003). Structure and function of ethnic skin and hair. *Dermatol Clin* 21**,** 595–600.

Sawada S, Araki S & Yokoyama K (2000). Changes in cold-induced vasodilatation, pain and cold sensation in fingers caused by repeated finger cooling in a cool environment. *Ind Health* 38**,** 79–86.

Serneri GGN, Masotti G, Gensini GF, Poggesi L, Abbate R & Mannelli M (1981). Prostacyclin and thromboxane A2 formation in response to adrenergic stimulation in humans: a mechanism for local control of vascular response to sympathetic activation? *Cardiovasc Res* 15**,** 287–295.

Spealman CR (1945). Effect of ambient air temperature and of hand temperature on blood flow in hands. *Am J Physiol* 145**,** 218–222.

Thompson-Torgerson CS, Holowatz LA, Flavahan NA & Kenney WL (2007). Cold-induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in human skin. *Am J Physiol Heart Circ Physiol* 292**,** 1700–1705.

Thomson ML (1955). Relative efficiency of pigment and horny layer thickness in

protecting the skin of Europeans and Africans against solar ultraviolet radiation. *J Physiol* 127**,** 236–246.

Tindall H, Tooke J, Menys V, Martin M & JA D (1985). Effect of dazoxiben, a thromboxane synthetase inhibitor on skin-blood flow following cold challenge in patients with Raynaud's phenomenon. *Eur J Clin Invest* 15**,** 20–23.

Tyler CJ, Reeve T & Cheung SS (2015). Cold-induced vasodilation during single digit immersion in 0°C and 8°C water in men and women. *PLoS One* 10**,** e0122592.

Ungley CC & Blackwood W (1942). Peripheral vasoneuropathy after chilling "immersion foot and immersion hand." *Lancet* 2**,** 447–451.

Ungley CC, Channell GD & Richards RL (1945). The immersion foot syndrome. *Wilderness Environ Med* 33**,** 17–31.

Vane JR (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 231**,** 232–235.

Vanhoutte PM (2011). Endothelium-dependent contractions in hypertension: when prostacyclin becomes ugly. *Hypertension* 57**,** 526–531.

Wrenn K (1991). Immersion foot. A problem of the homeless in the 1990s. *Arch Intern Med* 151**,** 785–788.

ADDITIONAL INFORMATION

COMPETING INTERESTS

Professor M J Tipton is editor-in-chief of Experimental Physiology.

AUTHORS CONTRIBUTIONS

The laboratory work of the present study was conducted at the University of Portsmouth. All authors contributed to the design of the research protocol; MJM collected and analysed data; all authors interpreted results of experiments; MJM prepared tables, figures and drafted manuscript; all authors edited and revised manuscript; all authors approved final version of manuscript; all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

FUNDING

This research was funded by the University of Portsmouth.

ACKNOWLEDGEMENTS

Thank you to the University of Portsmouth for funding the research. We also thank the participants for their time and commitment to this study.

TABLES

Table 1. Mean (SD) participant characteristics

	Participant Characteristics	
	CAU	**AFD**
Age (years)	20 (4)	20 (2)
Height (cm)	178.2 (6.9)	176.0 (7.9)
Mass (kg)	73.1 (12.3)	74.1 (12.8)
Hand Length (cm)	20.0 (1.1)*	21.2 (1.2)
Foot Length (cm)	26.3 (1.2)	27.0 (1.8)
Hand Volume (L)	0.42 (0.12)*	0.53 (0.13)
Foot Volume (L)	1.16 (0.19)	1.24 (0.32)

* Significant difference between CAU and AFD ($P < 0.05$). $n = 12$ for each group.

Table 2. Mean (SD) mean arterial pressure (mmHg) for the foot and hand protocol as an average of both ethnic groups in both placebo and COX inhibition trials

	Mean Arterial Pressure (mmHg)	
Time	**Foot**	**Hand**
Baseline	83 (7)	82 (8)
30 seconds cooling	88 (9)*	84 (9)
5 minutes cooling	87 (9)*	86 (9)
15 minutes cooling	84 (6)	85 (7)
30 seconds rewarming	85 (8)	83 (8)
5 minutes rewarming	82 (7)	82 (7)

* Significant difference compared with baseline ($P < 0.05$).

Table 3. Mean (SD) or median (IQR) toe and finger cutaneous vascular conductance (flux•mmHg^{-1}) and skin temperature (°C) at the end of warm water immersion for the foot and hand protocol following placebo or COX inhibition

	Baseline Cutaneous Vascular Conductance (flux•mmHg^{-1})			
	Placebo		COX inhibition	
Skin site	**CAU**	**AFD**	**CAU**	**AFD**
Toe	2.2 (1.7)	3.1 (1.1)	1.8 (1.5)*	3.4 (1.7)
Finger	4.7 (0.8)	4.8 (1.0)	4.3 (1.2)	5.1 (1.1)
	Baseline Skin Temperature (°C)			
Toe ^	35.1 (1.1)	35.6 (1.2)	34.8 (1.6)*	35.5 (0.6)
Finger ^	35.9 (0.4)	35.8 (0.2)	35.8 (0.2)	35.8 (0.1)

^ Median (IQR). * Significant difference between CAU and AFD ($P < 0.05$). $n = 12$ for each group.

Table 4. Mean (SD) or median (IQR) cutaneous vascular conductance (flux•mmHg^{-1}) variables for the toe skin site during foot cooling and rewarming following placebo and COX inhibition

		Cutaneous Vascular Conductance (flux•mmHg^{-1})			
		Placebo		COX inhibition	
		CAU	**AFD**	**CAU**	**AFD**
Cooling	**Average**	1.6 (1.7)	1.7 (1.4)	1.3 (1.3)	2.3 (1.6)
	Minimum ^	0.2 (0.6)	0.2 (0.4)	0.1 (0.2)	0.2 (0.7)
	AUC	49 (51)	55 (43)	40 (39)	70 (51)
Rewarming	**Average ^**	1.2 (2.8)	1.5 (2.9)	1.5 (2.3)	1.5 (3.6)
	Minimum ^	0.2 (1.2)	0.2 (0.6)	0.3 (0.3)	0.4 (2.3)
	AUC	25 (23)	27 (22)	25 (22)	32 (32)

^ Median (IQR). AUC = Area under the curve. $n = 12$ for each group.

Table 5. Mean (SD) or median (IQR) cold-induced vasodilatation variables for the toe skin site during the foot cooling protocol following placebo and COX inhibition

	Placebo		COX inhibition	
	CAU	**AFD**	**CAU**	**AFD**
Average T_{sk} (°C)	14.4 (3.4)	15.9 (4.1)	13.7 (3.1)†	16.4 (3.3)
Minimum T_{sk} (°C) ^	9.3 (4.1)	10.9 (7.8)	9.1 (2.4)	12.6 (6.6)
	CAU (*n* = 2)	**AFD (*n* = 4)**	**CAU (*n* = 2)**	**AFD (*n* = 7)**
Number of waves	1.0 (0.0)	1.5 (0.6)	1.5 (0.7)	1.6 (0.5)
Onset time (min)	19.3 (4.0)	3.6 (0.8)	4.8 (3.3)	4.9 (3.6)
T_{sk} prior to onset of CIVD (°C)	14.0 (5.1)	19.9 (1.8)	19.6 (0.7)	18.1 (2.4)
Max T_{sk} during CIVD (°C)	15.7 (5.2)	22.5 (2.1)	21.2 (1.4)	20.5 (2.0)
Amplitude (°C)	1.7 (0.1)	2.7 (0.4)	1.7 (0.6)	2.4 (1.6)

† $P = 0.052$. ^ Median (IQR). $n = 12$ for average and minimum T_{sk}.

Table 6. Mean (SD) cutaneous vascular conductance variables for the finger skin site during hand cooling and rewarming following placebo and COX inhibition

		Cutaneous Vascular Conductance (flux•mmHg^{-1})			
		Placebo		COX inhibition	
		CAU	**AFD**	**CAU**	**AFD**
Cooling	**Average**	2.1 (0.9)	1.4 (0.8)	2.3 (1.1)	1.6 (0.6)
	Minimum	0.8 (0.2)*	0.5 (0.1)‡	0.9 (0.5)	0.6 (0.3)
	AUC	66 (27)	44 (24)	74 (35)	50 (18)
Rewarming	**Average**	4.3 (1.0)*	2.8 (1.6)	4.3 (0.6)*	2.8 (1.0)
	Minimum	1.5 (0.6)	1.2 (1.1)	1.6 (0.8)*	0.9 (0.5)
	AUC	66 (16)*	43 (24)	66 (10)*	45 (16)

* Significant difference between CAU and AFD ($P < 0.05$). ‡ Significant difference between placebo and COX inhibition ($P < 0.05$). AUC = Area under the curve. $n = 12$ for each group.

Table 7. Mean (SD) or median (IQR) cold-induced vasodilatation variables for the finger skin site during the hand cooling protocol following placebo and COX inhibition

	Placebo		COX inhibition	
	CAU	**AFD**	**CAU**	**AFD**
Average T_{sk} (°C)	14.6 (2.3)‡	13.0 (3.0)	15.2 (2.5)	13.5 (1.9)
Minimum T_{sk} (°C)	10.7 (1.3)*	9.5 (1.4)	10.4 (0.9)*	9.5 (1.2)
	CAU (n = 7)	**AFD (n = 4)**	**CAU (n = 4)**	**AFD (n = 5)**
Number of waves ^	1.0 (1.0)	1.0 (0.0)	2.0 (1.5)	1.0 (0.5)
Onset time (min)	9.7 (6.2)‡	10.7 (8.5)	6.1 (5.9)	7.3 (5.6)
T_{sk} prior to onset of CIVD (°C)	13.8 (4.3)	14.1 (4.2)	15.8 (6.2)	14.9 (4.4)
Max T_{sk} during CIVD (°C)	17.5 (6.2)	19.1 (3.8)	19.3 (5.6)	18.2 (5.1)
Amplitude (°C)	3.6 (2.3)	5.0 (1.4)	3.5 (3.3)	3.3 (1.0)

^ Median (IQR). * Significant difference between CAU and AFD ($P < 0.05$). ‡ Significant difference between placebo and COX inhibition ($P < 0.05$). $n = 12$ for average and minimum T_{sk}.

FIGURE CAPTIONS

Figure 1. Schematic of the experimental procedure

Figure 2. Typical trace of finger skin temperature (°C) during hand immersion in water at 8 °C and subsequent rewarming in air at 30 °C showing variables analysed during cold-induced vasodilatation.

Figure 3. Mean (SD) toe cutaneous vascular conductance (flux•mmHg^{-1}) (a) and skin temperature (°C) (b) during foot cooling and subsequent spontaneous rewarming following placebo and COX inhibition

Note: SD included for CAU Placebo protocol only for reader clarity.

Figure 4. Mean (SD) finger cutaneous vascular conductance (flux•mmHg^{-1}) (a) and skin temperature (°C) (b) during hand cooling and subsequent spontaneous rewarming following placebo and COX inhibition

* Significant difference between CAU and AFD following placebo ($P < 0.05$). † Significant difference between CAU and AFD following COX inhibition ($P < 0.05$). Note: SD included for CAU Placebo protocol only for reader clarity.